

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Mark C. Fishman et al. Confirmation No.: 8749  
Serial No.: 10/656,873 Art Unit: 1634  
Filed: September 5, 2003 Examiner: Jehanne Souaya Sitton  
Customer No.: 21559  
Title: Methods for Diagnosing and Treating Heart Disease

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

DECLARATION OF MARK C. FISHMAN, M.D., AND XIAOLEI XU, PH.D.

UNDER 37 C.F.R. § 1.131

We declare:

1. We are the inventors of the subject matter that is described and claimed in the above-captioned patent application.
2. The enclosed Exhibit is a copy of laboratory notebook pages, which show that we determined that the pickwick mutation, which is characterized by a weak heartbeat, is in the titin gene. In particular, we found that certain zebrafish sequences that we had identified as being in the pickwick locus were homologous to known titin sequences. These pages are dated prior to the August, 1999 publication date of Satoh et al. (Biochem. Biophys. Res. Com. 262:411-417, 1999). This work was carried out in the United States of America.

3. All statements made herein of our own knowledge are true, and all statements made on information and belief are believed to be true, and further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: \_\_\_\_\_

\_\_\_\_\_  
Mark C. Fishman, M.D.

Date: \_\_\_\_\_

\_\_\_\_\_  
Xiaolei Xu, Ph.D.

pickwick

positional cloning

449

1803p marker use 800ng/well.

500

10789

(8 RE/1000)

→ 1 YAC

Connection

500 embryos

→ 3 YAC. by primers

from B'UTR. of connexin  
as superpool.

1

went on <sup>140</sup>use the primer pair to screen the 8 plate pool

Total 24 PCR reaction.

12  
8

-8

30 x 3 = 90

Genomic DNA 1 675 50X. 3 ~~8~~ 1/λ use 3λ

order

cos → enable.

35 cycle

x90

25 λ

Template

4 λ

(10 ng/λ)

26.

10X A

205 λ

225

25 mM dNTP.

0.1 λ

9

17.8

7

90

1602

→ 6.

primer

0.25 λ

20 mM

22.5

- primer

0.25

22.5

Tag

0.1 λ

9

H<sub>2</sub>O 4

17.8 λ

1602

25 λ

1. IVF fish do not give eggs out try  
next week. select strong fish!  
try with my m686 w/ fish although AB/TL  
background. at least. get something.

2.

Titin

picknick could be titin. zch1256 show high homology with  
titin (connection), which makes sense.

1. Z8363 scan all mutants to identify recombinants.

> 500 embryos. confirm with Z20031 about mid ID'd.

2. design primers from af 0361148. do ~~PCR~~ PCR

together with zch1256 against Y5, Y6. hope to pick  
up the right side about Y5T3, Y6T3.

3. compare human, mouse / chick / titin sequence. design  
primer pairs against the 27 kb cDNA conserved region

① put into RH map. confirm its identity.

② PCR against Y5, Y6.

③ isolate BAC. & get the intron. 3' UTR region

and then design primers for SSCP.

~~zch1256~~

148

1. Got embryos for m1062H (their parents are here)

m686.9 x TL07 (two pairs)

m1010H

m521A ( # are low)

mP18a (TL allele)

Today bleached five out of 6 except m521A  
next Tuesday put them into system.

Tomorrow look at phenotype

More good news!

1. from EST project. 4 were titin zebrafish version!

2. Y5T7 end ~~is~~ is titin homologue!

3. Best of all. they represent different portions of titin

2.9K 3.2 5.2  
A158854 A160182  
T1 A1588106 T3  
T2

2.4 2.6 2.5 2.7  
Y5T7 A1353993  
T4 A1629069  
Zeb1256 T5

4. according to sequence alignment of Y5T7 the titin gene in chromosome should be

28363

0

Zeb1256

24K

27K

titin

Y5T3 ←

Y5T7

WITNESS:

DATE: